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Correlation of Maternal and Pup NK-Like Activity and TNF Responses Against Cytomegalovirus to Pregnancy Outcome in Inbred Guinea Pigs

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Immunologic causes for poor outcome of pregnancy complicated by primary cytomegalovirus (CMV) infection are only partially understood. Maternal and pup tumor necrosis factor (TNF) and natural killer cell (NK)-like activity associated with primary gestational CMV infection initiated in either the first or third trimester equivalent in the inbred guinea pig model were investigated. Poor pregnancy outcome defined as fetal resorptions, premature delivery, stillbirths, and intrauterine growth retardation occurred with infection at either gestational time. Induction of TNF and NK activity by CMV infection during pregnancy correlated with resorptions in early pregnancy infection and with premature labor in late pregnancy infection. Stillbirths occurred with CMV infection at either time. Regardless of the gestational time of CMV acquisition, poor outcome correlated with higher maternal NK and TNF responses during the first weeks after maternal virus acquisition. Furthermore, CMV infected dams with loss of $\geq$ 50% of conceptus had higher TNF responses than infected dams with < 50% conceptus loss. Likewise, pups born in litters from CMV-infected dams with resorptions and/or premature labor also had enhanced NK activity and TNF response to CMV compared with pups born to dams not having resorptions or premature labor. TNF responses in the delivered pups of infected dams were higher than from pups of uninfected dams regardless of litter outcome, whereas pup NK responses were enhanced only in pups from litters of dams with premature labor or resorptions. Enhanced NK and TNF activity appear to be associated with premature delivery and other poor outcomes of pregnancy. ***J. Med. Virol. 60:230–236, 2000.***

KEY WORDS: CMV; NK; TNF; pregnancy outcome; maternal; animal model or guinea pig

INTRODUCTION

Congenital cytomegalovirus infection is associated more frequently with disease after maternal primary compared with recurrent/reactivation infection during pregnancy [Fowler et al., 1992]. Acquired maternal immunity is an important factor in the reduced frequency and severity of CMV infection of the fetus. The quantity and duration of maternal viremia, the pathway of virus to the fetus, may be critical factors in the likelihood of fetal infection and outcome. In the animal model, a decreased maternal viral load occurring in recurrent or reactivated infection in contrast to primary maternal infection in pregnancy, seems to be one marker of improved pregnancy outcome [Griffith et al., 1990; Harrison and Myers, 1990]. Less intense maternal viremia was also a marker for better fetal outcome after primary maternal infection when the dams had been immunized prior to viral challenge with a subunit gB vaccine [Harrison et al., 1995]. A particular benefit seems to accrue if maternal intravascular viral load is reduced. This benefit may result from reduced viral density at the maternal–fetal interface, i.e., the placenta, before complete cessation of maternal viremia

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occurs. For this postulate to be correct, direct viral effects on placenta and fetus would need to be the major pathologic mechanism in disease due to congenital CMV infection. It would also be logical that strategies to enhance maternal CMV immunity and reduce viral load, particularly in the blood, should reduce placental or fetal consequences [Fowler et al., 1992]. However, it is also possible that not all enhanced maternal responses are beneficial to the placenta/fetus despite their protective effects for the mother.

Because ethical considerations and practical difficulties limit the investigations into such aspects of congenital CMV infection during human pregnancy, investigations of maternal CMV infection are facilitated by animal models. Guinea pig and human placentas are similar, both allowing congenital infection [Kaufmann, 1977]. The relatively long (65–70 days) guinea pig gestation also exhibits trimester-like divisions. During gestational primary guinea pig cytomegalovirus (GPCMV) infection, maternal viremia persists longer than in nonpregnant controls [Harrison and Myers, 1990], and also has been reported to recur [Griffith et al., 1990]. Like humans, intrauterine transmission occurs in ~30% of offspring after maternal primary GPCMV gestational infection [Harrison and Myers, 1989], with 5–10% central nervous system infections. Intrauterine growth retardation (IUGR) and 40–60% loss of conceptus via fetal resorptions or stillbirths also occur after maternal primary GPCMV infection. The fact that IUGR and stillbirths occur even in uninfected pups from litters of dams undergoing primary GPCMV infection, in fact suggests that fetal/placental damage does, at least in some cases, occur by mechanisms other than direct virus-produced damage. Alternatively, the pathology may be sequelae of a transient placental infection, cleared after some time by maternal or fetal immune mechanisms.

Here we report an investigation into two immunological maternal responses in the inbred JY-9 guinea pig model. The first is cytolytic cell-mediated NK-like activity against GPCMV-infected cells that might reduce maternal viral load and/or directly damage the placenta. The second is in vitro TNF production by peripheral blood mononuclear cells (PBMC) in response to GPCMV antigen, that might affect fetal nutrition, development, viability, and/or birth size by one of several mechanisms: (1) Maternal mechanism (provoking premature labor); (2) interface mechanism (producing placental injury); or (3) fetal effect (fetal tissue injury after passive transfer of TNF from the maternal system).

METHODS

Tissue Cultures and Virus

GP lung fibroblast (GPL) cultures were derived from a Strain-2 guinea pig cell line. GPCMV (strain no. 22122, American Type Culture Collection [ATCC] #VR682, Rockville, MD) was used to prepare salivary gland-derived (SG) GPCMV stock of moderate virulence by seven sequential passages in vivo in salivary glands. The clarified homogenate was stored at 1.0×10^6 PFU/mL until used [Harrison and Burger, 1991].

Animals

Inbred JY-9 guinea pigs were obtained from the Creighton Vivarium (Omaha, NE). Pregnant dams were inoculated SQ with SG-GPCMV (5×10^4 PFU) on day 17–21 (Early) or day 48–51 (Late) of pregnancy. Four pregnant dams of each group (early or late) were also inoculated with mock-virus (clarified homogenate of salivary glands processed identically as above but obtained from uninfected animals) on the same schedule.

Pregnancy Outcome

Twelve dams were inoculated subcutaneously with GPCMV during early gestation, but 3 died with fulminant maternal hepatitis within 10 days of inoculation, leaving 9 dams for evaluation. Resorptions were documented by abdominal palpation, enumerating fetuses on day 25–30 and day 40–45 of gestation and comparing with the number delivered [Harrison and Myers, 1989, 1990] as stillborn plus live-born. Birth weight was documented for delivered pups, as was duration of gestation. Delivery was defined as premature if it occurred before 60 days of the normal 67–72-day gestation. This time frame would approximate < 36 weeks of human gestation.

NK-Like Cytotoxicity Assays

Uninfected and GPCMV-infected (multiplicity of infection (MOI) of 1 for 48 hr) Strain-2 GPL were used as targets in standard ^{51}Cr release assays [Bernstein et al., 1991]. Pup splenocyte or maternal PBMC effectors (3.2×10^5) were obtained and incubated for 14 hr with ^{51}Cr-labeled targets (4×10^3) yielding an 80:1 E:T ratio. E:T supernatants were counted for CPM and % lysis calculated. Major histocompatibility complex (MHC)-unrestricted GPCMV-specific cytolysis (NK) was defined as the % lysis of GPCMV-infected GPL minus that of uninfected GPL.

GPCMV Antigen

Three 175-cm^2 flasks of confluent JY-9 Strain-derived fetal guinea pig cells in BME with 10% fetal calf serum (FCS) and antibiotics were inoculated with GPCMV (strain no. 22122, ATCC #VR682) at an M.O.I. of 10:1. When cytopathic effect was noted to be maximal (7 days), cells were harvested, washed three times in phosphate-buffered saline (PBS), centrifuged, and restored to a 3 ml volume with PBS. The pellet was freeze thawed three times and sonicated three times for 15 sec. Mock-infected fetal guinea pig cells were also prepared in the same fashion as control antigen [Bernstein et al., 1991]. Antigen was used at a concentration of 25 μg/ml.

PBMC and Splenocyte Preparation

As reported previously, PBMC were obtained by Ficoll-hypaque centrifugation of citrated whole blood

TABLE I. Outcome of Pregnancy by Experimental Group of Dam (Early or Late Pregnancy Inoculation of Challenge Material, i.e., GPCMV Virus or Mock Inoculum)

Dam group	Dam total	Premature labor in dam	Pup total	Resorbed pups	Stillborn pups	Live-born pups	Birth weights	% Loss of conceptus
Mock early	4	0	13	0	1	12	100 ± 7	6.3
Mock late	4	0	14	0	0	14	101 ± 6	0.0
GPCMV early	9	1	28	5[a]	9[a]	14	82 ± 8[a]	50.0[a]
GPCMV late	9	7[b]	26	0	14[a]	12	89 ± 6[a]	53.8[a]

GPCMV, guinea pig cytomegalovirus.
[a]$P < .01$ vs. Mock Infected.
[b]$P < .02$ vs. all other groups.

[Harrison and Myers, 1989] and splenocytes were obtained by passing fetal spleens through sterile metal screen meshes into heparinized PBS followed by ammonium sulfate lysis of red blood cells [Harrison and Myers, 1989].

TNF Assays

After 2 days of culture of 5×10^5 maternal PBMC (or splenocytes from pups) in triplicate with GPCMV antigen or control antigen or 16 μg CON A, 100 μl of supernatant were removed for assay. TNF was assayed using the L929 bioassay with murine TNF-α (kind gifts from John Shanley, New Haven, CT) assayed in parallel as control. Briefly, 50 μl of supernatant was added to L929 cell monolayers in 96-well plates and 2-fold (1:2–1:256) serial dilutions were performed. Actinomycin D was added and plates incubated for 18 hr at 37°C in 6% CO_2. Endpoints were defined as > 50% reduction in cell density. The limit of sensitivity of the assay is 2–5u murine TNF-α. GP TNF is expressed in murine TNF-equivalent units.

Fetal/Pup GPCMV Infection

Fetal/pup GPCMV infection was defined by tissue culture of half fetuses when fetuses were too small and of fetal or newborn individual organs when large enough, and by polymerase chain reaction (PCR) of the same tissues extracted by conventional phenol/chloroform methods and amplified with gB primers as reported previously [Harrison et al., 1995].

Statistics

Sigmastat statistical package was used for analysis with proportions analyzed by Chi-square or Fisher exact depending on expected cell size. Means were analyzed by analysis of variance (ANOVA). Two-tailed testing was used in all analyses when necessary. Cytolytic data were transformed before analysis. Significance was assigned to $P < .05$.

RESULTS

Pregnancy Outcome (Table I)

Fetal resorptions. After first trimester (Early) GPCMV challenge, one fetal resorption was observed in five of nine pregnancies compared with none in the Early Mock-challenged pregnancies and none in litters of Late-challenged dams. There were no resorptions in four of the Early GPCMV-challenged litters. The total resorption rate for all nine litters from Early GPCMV-challenged dams was 18% (5/28). If only dams with resorption are analyzed ($N = 5$ in Early group only), the resorption rate was 29% (5/17). In contrast, there were no resorptions in the groups of Early and Late Mock-challenged dams and in the group of Late GPCMV-challenged dams.

Stillbirths and birth weight. Nine additional pups (32.1%) of Early GPCMV-challenged dams were stillborn compared with 1/13 (7.7%) pups from Early Mock-challenged dams, $P < .05$. Likewise, 14/26 pups (53.8%) of Late GPCMV dams were stillborn compared with none of 13 pups from Late Mock-challenged dams, $P < .01$. The live-born rate in Early GPCMV-challenged dams was 50% (14/28) and in Late-challenged dams was 46.2% (12/26). Mean birth weight was lower in the 23 pups of Early GPCMV dams than in the 13 pups of MOCK Early dams (82 ± 8 g vs. 100 ± 7 g, $P < .02$). Mean birth weight was also lower in 26 pups of Late GPCMV than in the 14 pups of Mock Late dams (89 ± 6 g vs. 101 ± 6 g, $P < .05$).

Premature labor. The mean length of gestation for the nine Early GPCMV-challenged dams that survived to delivery was 65.8 ± 3.9 days compared with 69.0 ± 1.5 days (not significant) for the Early Mock-infected dams. After Late (third trimester) GPCMV challenge, premature labor was observed in seven of nine dams (mean gestation 57.8 ± 2.6 days compared with Mock Late 69.8 ± 3.1 days, $P < .05$).

GPCMV NK-Like and TNF Activity in Dams

NK-like activity against GPCMV targets (Fig. 1) and in vitro TNF response to GPCMV (Fig. 2) were elevated at 7–21 days after virus infection, in both Early- and Late-challenged dams, compared with their Mock-infected counterpart dams. NK-like activity in dams with > 50% conceptus loss was increased compared to those with < 50% loss only on day 7 postinfection, 31.4 ± 6.9% vs. 22.7 ± 8.3%, $P < .05$. The GPCMV infected pregnant dams from both Early and Late groups that had > 50% pup losses due to the resorptions and/or premature deliveries ($N = 5$ in early group plus 4 in late group) also had higher TNF responses compared to infected dams with ≤ 50% pup loss ($N = 4$ in early group plus 5 in late group), $P < .001$ to $P < .05$ (Fig. 3). The highest NK-like activity against GPCMV targets was noted in the Late GPCMV-challenged dams on day 21 postinfection (Fig. 1). This was an average of ~8 days

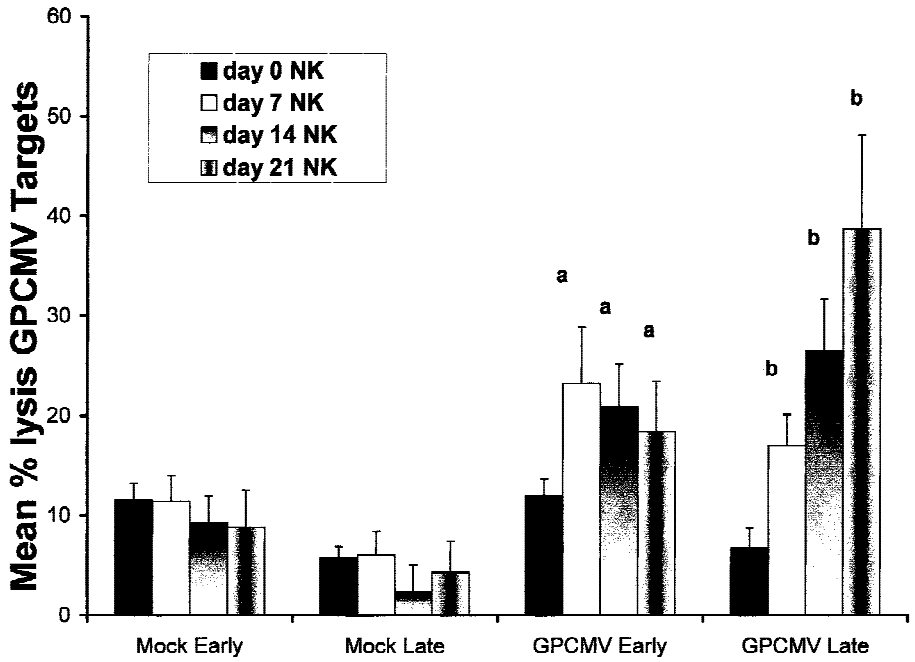

Fig. 1. Maternal natural killer cell-like (NK) activity against 4×10^3 guinea pig cytomegalovirus (GPCMV)-infected fibroblast targets in 3.2×10^5 peripheral blood mononuclear cells (PBMC) from dams on days 0, 7, 14, and 21 after inoculation. Dams were inoculated on day 0 with 5×10^4 PFU of salivary gland-derived virus (GPCMV) or salivary gland extract without virus (Mock virus) subcutaneously. Early or Late refers to the time of gestation when GPCMV or Mock inoculation was performed. Early denotes inoculation in the first trimester equivalent and Late denotes inoculation in third trimester equivalent of guinea pig pregnancy. The percent cytolysis values represent net GPCMV specific cytolysis calculated by subtracting background cytolysis of uninfected fibroblasts from cytolysis of GPCMV-infected fibroblasts. a = $P < .01$ vs. Mock Early. b = $P < .001$ vs. Mock Late.

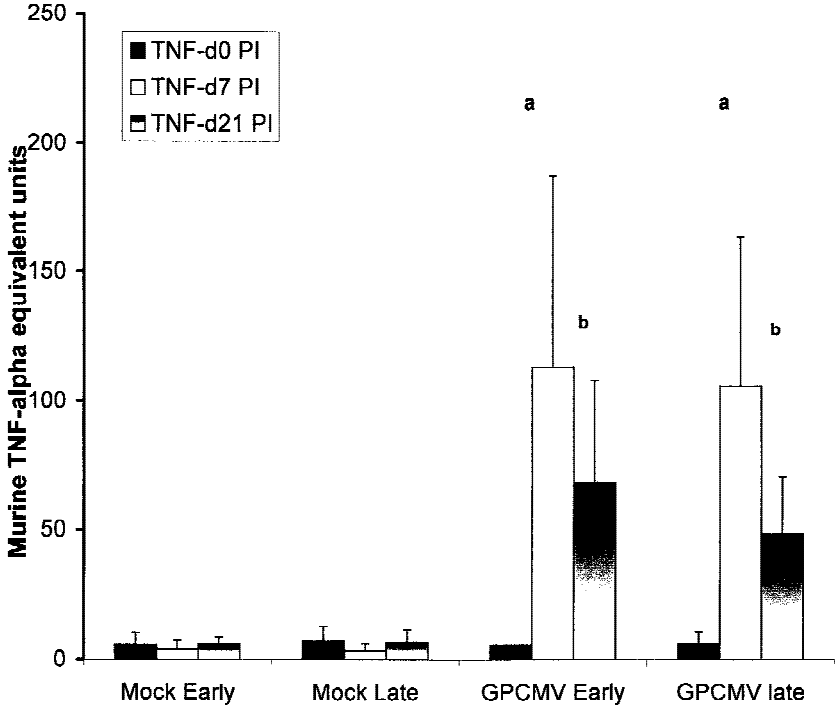

Fig. 2. Maternal in vitro tumor necrosis factor (TNF) responses to guinea pig cytomegalovirus (GPCMV) whole antigen (triple freeze-thawed and sonicated lysate of fibroblasts GPCMV-infected for 7 days) by 5×10^5 peripheral blood mononuclear cells (PBMC) expressed in murine TNF-alpha equivalent units. PBMC were from dams on days 0, 7, and 21 after inoculation with 5×10^4 PFU of salivary gland-derived virus (GPCMV) subcutaneously. Early or Late refers to the time of gestation when GPCMV inoculation was performed. Early denotes inoculation in the first trimester equivalent and Late denotes inoculation in third trimester equivalent of guinea pig pregnancy. a = $P < .001$ vs. Mock Groups. b = $P < .01$ vs. Mock Groups.

after delivery. The in vitro TNF response to GPCMV antigen, however, lessened on this day and did not parallel the ongoing rise in NK-like activity.

Pup Data (Table II)

Ten of 23 delivered pups (28 total pups minus the 5 resorbed fetuses, which could not be tested) from the early GPCMV-infected dams were infected congenitally as determined by culture or PCR, resulting in a 35.7% for all pups and 43.5% rate for delivered pups. These rates may be misleading because some of the 5 resorbed pups may have been infected but were not available for assays. Nine of 26 pups from the late GPCMV-infected dams were infected congenitally, for a rate of 34.6%.

Pup immunological data were analyzed by litters based on the effects of the infection on the dams. Infected dam effects were grouped by presence of resorptions, premature labor, or neither. In the pups from Mock-infected groups, GPCMV NK-like activity was

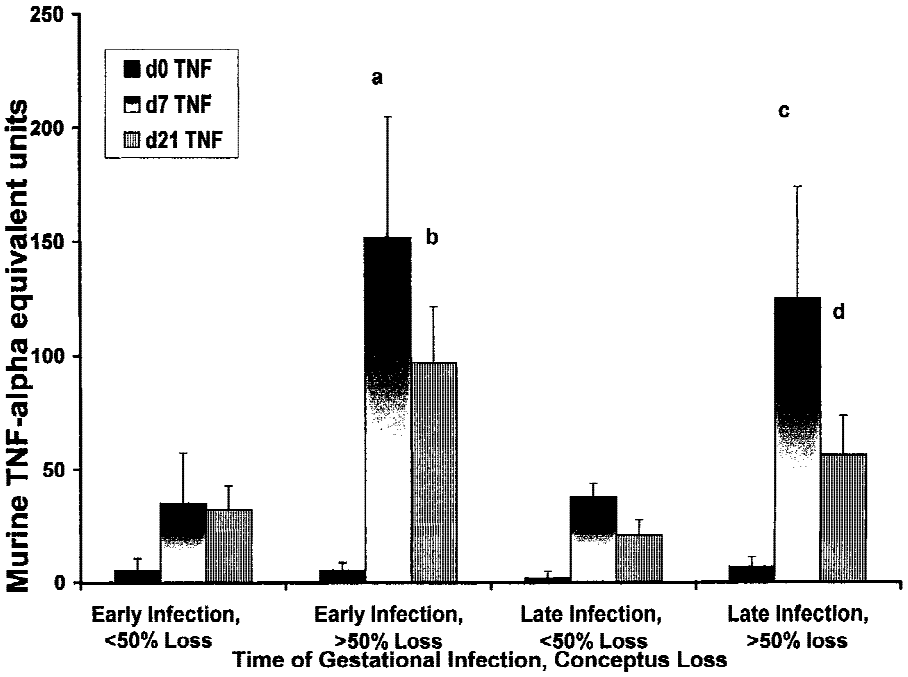

Fig. 3. Maternal in vitro tumor necrosis factor (TNF) responses to guinea pig cytomegalovirus (GPCMV) whole antigen (triple freeze-thawed and sonicated lysate of fibroblasts GPCMV-infected for 7 days) by 5×10^5 peripheral blood mononuclear cells (PBMC) expressed in murine TNF-alpha equivalent units. PBMC were from dams on days 0, 7, and 21 after inoculation with 5×10^4 PFU of salivary gland-derived virus (GPCMV) subcutaneously. Early or Late refers to the time of gestation when GPCMV inoculation was performed. Early denotes inoculation in the first trimester equivalent and Late denotes inoculation in third trimester equivalent of guinea pig pregnancy. Dams were grouped by whether there was < or ≥ 50% conceptus loss within a litter by way of resorption or stillborns within the GPCMV inoculated maternal groups. a = $p < 0.001$ compared to Early < 50% Loss. b = $p < 0.01$ compared to Early < 50% loss. c = $p < 0.005$ compared to Late < 50% Loss. d = $p < 0.05$ compared to Late < 50% loss.

TABLE II. NK-Like Activity Against GPCMV Targets in Delivered-Pup Splenocytes and In Vitro TNF Response to GPCMV Antigen From Pup Splenocytes Obtained Within 48 hr of Delivery

Origin of pup litters	Pups of early infected dams, $N=$	Pups of late infected dams, $N=$	Assayed pups infected/ uninfected	% Cytolysis NK GPCMV	In vitro TNF (units)	Pup birth weight (g)
Pups of mock infected dams[a]	6	6	0/12	8 ± 2	≤9	101 ± 7
Pups from infected dams (resorptions, no premature labor)	8	—	6/8[b]	19 ± 6[b]	59 ± 18[b]	68 ± 11[b]
Pups from infected dam (no resorptions, premature labor)	—	11	8/11[b]	29 ± 5[b]	46 ± 14[b]	74 ± 9[b]
Pups from infected dam (no resorptions, no premature labor)	9	6	6/15[c]	11 ± 9[d]	27 ± 11[c]	87 ± 6[c]

NK, natural killer cells; GPCMV, guinea pig cytomegalovirus; TNF, tumor necrosis factor.
[a]No significant difference between results from pups born to Early and Late Mock Challenged Dams, therefore data combined. The spleen from one pup was inadvertently contaminated preventing its use in assays.
[b]$P < .01$ vs. Mock Infected.
[c]$P < 0.02$ vs. pups from other three groups, i.e., from litters with maternal Mock infection, or maternal GPCMV infection plus resorptions, or maternal GPCMV infection plus premature labor.
[d]$P < .02$ compared to other two pup groups from infected dams. No difference compared to Mock infected group pups.

lower than in the five live-born plus three stillborn pups of the GPCMV-challenged dams that had resorptions. GPCMV NK-like activity was also lower in these pups from Mock-infected dams than in eight live-born and three stillborn pups of the GPCMV-challenged dams that had premature labor. However, pups born to GPCMV-infected dams without either resorptions or premature labor had similar NK-like activity against GPCMV targets to pups born to Mock-infected dams (Table II). NK-like activity did not differ significantly between the 20 congenitally infected pups and the 46 uninfected pups (data not shown).

Compared with pups from Mock-infected pregnancies, in vitro TNF response to GPCMV antigen by pups from GPCMV-challenged pregnancies without premature labor or resorptions was higher than pups from Mock-infected dams and lower than pups from litters whose dams had experienced resorptions or premature labor (Table II).

DISCUSSION

The nonpregnant immunocompetent guinea pig undergoing initial GPCMV infection has either asymptomatic seroconversion or a mononucleosis syndrome similar to immunocompetent humans with primary CMV infection. With CMV infection during pregnancy, humans and guinea pigs have similar rates of transmission, variable transmission within multiple fetuses of a single pregnancy, and similar organ involvement with variable disease expression in the congenitally infected newborns. This similarity is likely due to similar tropism of each species CMV, the similarity in placental structure [Davidoff, 1973], and the relatively long duration of a guinea pig 70-day pregnancy that allows

trimester-like divisions. Thus, mechanisms of injury defined in this model are reasonable bases for the pathogenesis of congenital CMV disease in humans. For example, human CMV is involved in placentitis and fetal loss in the first trimester, as our data show to be the case also with GPCMV in the guinea pig. Maternal cell-mediated functions are suppressed in both normal human and normal guinea pig pregnancy, but the guinea pig cell-mediated arm is enhanced with production of pro-inflammatory cytokines such as TNF-α with GPCMV infection during early and late pregnancy. These immune enhancements appear needed to contain the infection in the mother guinea pig, but also are associated with poor pregnancy outcome (abortion, premature labor, IUGR). And despite these responses, congenital infection takes place in similar proportion of fetuses if they survive 2 weeks after the initial maternal illness.

The mammalian maternal–fetal unit is a complicated interacting system with the maternal side being the usual access for extraneous influences on the fetus via the placental interface or ascending via the birth canal to the chorioamniotic sac. Maternal immune functions can be helpful to the fetus (passive antibody to neutralize an enterovirus [Modlin, 1980]) or detrimental (excess immune recognition leading to spontaneous abortion [Rocklin et al., 1976]). Furthermore, maternal immune responses include innate functions such as NK-like cells, which can attack and lyse CMV-infected cells when recognized as non-self. In addition, cytokines (such as interferon-α [IFN-α] or TNF), which impair cell replication, growth, and may lead to apoptosis, may be detrimental in the triggering of premature labor or in the direct effects on rapidly growing tissues in the placenta or fetus. These cytokines have direct effects on both CMV-infected and nearby uninfected bystander cells. Pro-inflammatory cytokines also have the capacity to potentiate nonspecific killing of cells, CMV-infected or not, by cytokine-activated mononuclear cells, potentially leading to additional injury of uninfected bystander and CMV-infected placental or fetal cells.

When maternal pro-inflammatory immune mechanisms are activated during pregnancy, NK-like activity could reduce maternal viral load, but at the same time damage placental tissue in the process of lysing CMV-infected cells at the interface and/or in vascular endothelium. Likewise, soluble factors such as TNF or IFN-α could affect placental and even fetal tissue maintenance or restrict growth [Dammann and Leviton, 1997; Haddad et al., 1997; Argiles et al., 1998] if elicited in sufficient amounts. Thus, maternal immune response could protect the fetus by restricting viral load, or it could compound direct viral damage to the placenta by attacking viral-infected cells at the maternal–fetal interface or leaching soluble factors into or across the placenta.

Finally, the effects of any combination of the direct damage due to viral infection, action of cytokines, or injury from cell-mediated immune responses could reduce the function of the placenta. Altered function or perhaps even lessened viability of the placenta could reduce growth or viability of the fetus, or cause placental separation, even if transplacental infection is prevented or reduced by the immune responses. Credence to this possibility arises from reports that certain cytokines, particularly TNF, have been associated with premature labor during maternal bacterial infections such as urinary tract infections or amnionitis [Argiles et al., 1997; Imseis et al., 1997].

This model has demonstrated previously that premature labor, fetal resorptions, and stillborns are frequent after primary gestational GPCMV infection [Harrison and Myers, 1989; Bratcher et al., 1995]. As might be expected, fetal resorptions were observed after early but not late gestational GPCMV challenge. However, birth weights were reduced in delivered pups after gestational GPCMV infection regardless of the time of gestational challenge. We postulate this reduction in birth weight is due to direct and indirect effects of the infection.

The direct effects are the injury to the placenta by the virus itself, affecting the viability of the pregnancy with varying outcome depending on the severity of the infection and the time during gestation. This is exemplified by the outcome difference of premature labor plus IUGR in late pregnancy (third trimester equivalent) infection compared with resorption and smaller placentas in early pregnancy (first trimester equivalent) infection.

Indirect effects would include the effects of NK-like cells and TNF on all tissues whether infected or not in the evolving placenta/fetal unit. We postulate that these inflammatory responses also affect growth and even viability of the placenta and/or fetus in proportion to the responses. These responses could explain the IUGR and small placentas and lesser birth weights even from uninfected fetuses. The human equivalent might be first trimester abortions in early pregnancy infection and runted IUGR infants in late pregnancy infection. It is interesting to postulate that these outcomes might occur in some fetuses or infants that escape congenital infection but have been exposed to the maternal milieu of primary infection, if the maternal response is sufficiently robust.

It is not clear why some dams in an inbred animal strain receiving the same inoculum at the same time during pregnancy would have differing responses. We have shown that there is variable innate NK-like activity within this inbred strain [Harrison and Myers, 1989] and that it is suppressed as pregnancy progresses until just before delivery when it returns to normal. We postulated that this effect was due to pregnancy-associated hormonal changes. Maternal or placental hormonal influences may be possible factors.

Having defined associations does not prove causality between fetal/newborn injury and the maternal immune responses (NK-like activity and TNF-α), so interventions that block, reduce, or neutralize these responses would be useful in causality confirmation. Use

of anti-TNF antibody or low dose corticosteroids to reduce NK-like responses could be studied.

Furthermore, it remains unclear why some fetuses within a single pregnancy of humans or guinea pigs become infected or not and if infected become damaged or not. Likely explanations include differences in placental viral load, fetal immune responses, or eventual placental injury with alteration in the placental function. Additional studies are needed to confirm the placental affects of alterations in maternal and fetal TNF and NK responses.

Passive transfers of CMV antibody or CMV-immune cell subpopulations to the dam could define whether specific downstream immune responses are helpful or harmful to the placenta and/or fetus. The goal is to define the pathogenesis of congenital CMV disease such that interventional trials in humans could be designed. These studies could define protective from detrimental maternal responses that could provide benchmarks for vaccine candidates.

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